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Practical Guide to the Diagnosis of Thalassemia

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Thalassemias occur in individuals of all ethnic backgrounds and are among the most common genetic diseases worldwide. The diagnosis of thalassemia can easily be part of primary medical practice. Here we outline a practical approach to the detection of thalassemias in three common clinical settings. The first involves any patient with a low mean corpuscular volume (MCV) with or without anemia. The second is a neonatal screening result indicating possible presence of thalassemia. Finally, evaluation for thalassemia should be considered in the context of family planning or pregnancy in patients whose ethnicity indicates origin from high risk geographic areas. We also review the various types of the thalassemia syndromes and provide an overview of general therapeutic considerations.

KEY WORDS: thalassemia, microcytosis hemoglobinopathy, neonatal screening, prenatal diagnosis, anemia

INTRODUCTION

The thalassemias are a heterogeneous group of genetic disorders characterized by the absent or decreased production of normally functioning hemoglobins. Normal red blood cells contain a combination of 3 hemoglobins, A, A_2, and F (Table I). Each of these hemoglobins is composed of 2 α and 2 non-α (β, δ, or γ) globin chains. There are two α globin genes on chromosome 16, providing 4 copies in normal individuals. The γ, δ, and β globin genes are on chromosome 11.

Normally, the production of α and non-α chains is balanced. In the α thalassemias, the formation of α chains is decreased. This decrease is usually due to a deletion of 1 to 4 of the α globin genes. Some point mutations (i.e., Hb Constant Spring) also cause a decrease in the amount of α chains produced. In the β thalassemias, many point mutations or small deletions affect expression of the β globin gene and lead to decreased production of β chains. In both α- and β-thalassemia, the decreased amount of globin chains causes microcytosis, and the imbalanced synthesis results in ineffective erythropoiesis and hemolysis. This combination results in varying degrees of microcytic hypochromic anemia. Interactions among α thalassemia, β-thalassemia, and structural hemoglobin variants (i.e., Hb S, C, E, etc.) cause marked variations in clinical manifestations and complex inheritance patterns.

Thalassemia occurs in individuals of all ethnic backgrounds and is one of the most common genetic diseases worldwide. The thalassemia mutations occur in very high frequency in areas of past endemic falciparum malaria, including West and Central Africa, the Mediterranean basin, the Mi ddle East, South Asia, Southeast Asia, Southern China, and the Pacific Islands. These genes are also found commonly in other geographic areas due to population migration. Because of the diverse ethnic background of peoples in the United States, thalassemia may be detected in any medical practice; the clinician cannot solely rely on ethnic history when considering the diagnosis of thalassemia. Emigration of persons from areas of high frequency continues to increase the prevalence of these disorders in this country.

Although iron deficiency is the most common cause of acquired microcytosis, thalassemia is the most common form of inherited microcytosis. Patients with thalassemia syndromes present with manifestations that vary in severity from asymptomatic, mild microcytosis to a severe, life-threatening anemia. Treatment of the severe forms of thalassemia prolongs life and improves quality of life. Because iron overload is a significant

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TABLE I. Normal Hemoglobins

Hemoglobin	Globin chains
Hb A	$\alpha_2\beta_2$
Hb A_2	$\alpha_2\delta_2$
Hb F	$\alpha_2\gamma_2$

problem in all thalassemia syndromes, recognition of even mild forms prevents inappropriate administration of iron. The diagnosis of thalassemia can easily be part of primary medical practice. The purpose of this paper is to outline a practical approach to the detection of thalassemia in common clinical settings: (1) evaluation of low MCV; (2) neonatal screening and diagnosis of thalassemia during infancy; and (3) pregnancy and family planning.

APPROACH TO DETECTION AND DIAGNOSIS OF THALASSEMIA

Evaluation of the Low Mean Corpuscular Volume

Early detection of thalassemia can easily be part of routine and good medical practice. A low MCV, as measured by an electronic cell counter, is often the first or only clue to the presence of thalassemia. Therefore, when clinicians encounter a low MCV when interpreting a complete blood count (CBC) obtained for any purpose, thalassemia should always be considered. Figure 1 indicates how to use the MCV and other readily available tests to establish the diagnosis of thalassemia in individuals over age 1 year. The approach to evaluating a low MCV during pregnancy is different (Figs. 2, 3).

During infancy, the diagnosis of thalassemia is complicated by rapid physiologic changes in both red cell size and hemoglobin synthesis, which cause wide variation in both MCV and hemoglobin levels among individuals (Table II). In α thalassemia, microcytosis is often present from birth, while, in β thalassemia it typically develops later during infancy. The presence of fast migrating bands (Hb Bart's) on hemoglobin electrophoresis at birth can provide a unique opportunity to diagnose α thalassemia because the electrophoretic pattern of hemoglobin in mild forms of α thalassemia typically becomes normal after the first few months of life. The diagnosis of β thalassemia, which is often dependent on the presence of elevated levels of fetal (Hb F) and A_2 (Hb A_2) hemoglobins, may be problematic because of the physiologic variation in these hemoglobins present during infancy. Thus, diagnosis of β thalassemia syndromes may require family studies or repeated evaluation during the first years of life.

Neonatal Screening and Diagnosis of Thalassemia During Infancy

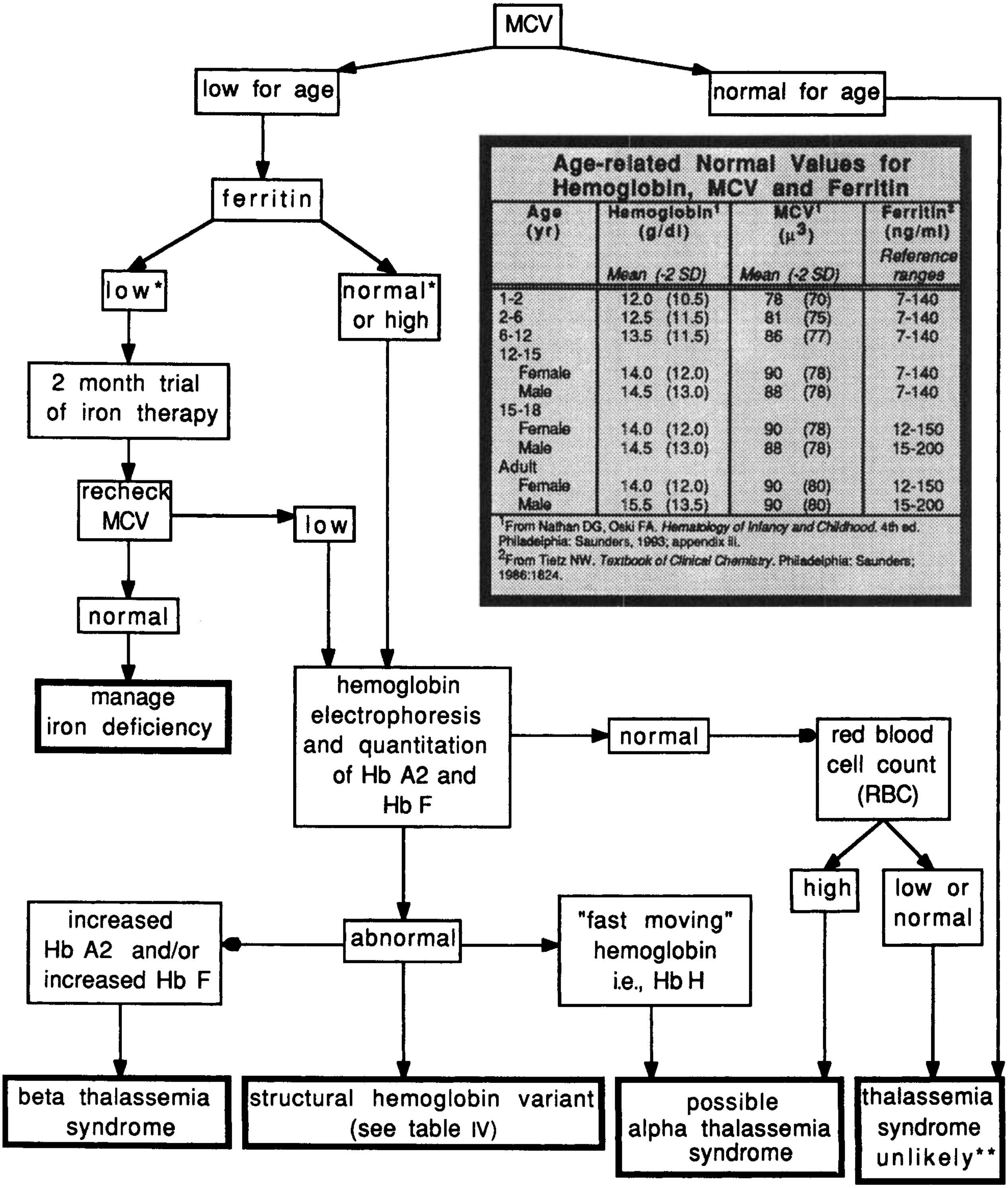
Neonatal screening for hemoglobinopathies may sometimes yield evidence of thalassemia (Fig. 4). High levels of an abnormally fast migrating hemoglobin band (i.e., Hb Bart's) on neonatal hemoglobin electrophoresis suggest Hb H disease (deletion of 3 of the 4 α globin genes). Infants with this finding require evaluation with a CBC and repeat hemoglobin electrophoresis. Family studies should be done as soon as is practical. To help diagnose α thalassemia in infants with low amounts of Hb Bart's on the newborn screening test, it may be necessary to repeat the hemoglobin electrophoresis and CBC at age 6 months. If this repeat hemoglobin electrophoresis no longer shows the presence of Hb Bart's and the MCV is low, the child likely has α thalassemia trait (2 α globin gene deletion), and family studies should be considered. If the "fast-moving" hemoglobin band persists on repeat electrophoresis (and the MCV is normal), the band is probably not Hb Bart's, and evaluation for a structural hemoglobin variant may need to be pursued. Infants with Hb Constant Spring should be evaluated with CBCs and family studies.

Newborn infants with "Hb F only" (absence of Hb A), Hb E, Hb Lepore, or another structural hemoglobin variant (e.g., Hb S or Hb C) in greater concentration than Hb A need evaluation with repeat hemoglobin electrophoresis and a CBC at age 2 to 3 months. In these cases family studies should be considered. "Hb F only" can be found in some normal infants (especially premature infants), but may also indicate a severe β thalassemia. Hb E homozygotes typically develop microcytosis with little or no anemia. Compound heterozygotes of Hb S, C, E, or Lepore and β thalassemia may develop significant anemia and other clinical problems. Because of previously discussed limitations in diagnosis of thalassemias in the first 6 months of life, early family studies may be very helpful and important in the evaluation of the infant. Repeated evaluation of the infant may also be required to define the clinical significance of the abnormal hemoglobin electrophoresis result.

Pregnancy and Family Planning

In light of the genetic transmission of the thalassemia disorders, detection and education of thalassemia carriers have important implications for pregnancy, genetic counseling, and family planning. Parents who are asymptomatic carriers of thalassemia and their relatives may be at risk of having severely affected offspring. These couples at risk may benefit from being identified so that they can be offered genetic counseling. Prenatal testing for thalassemia is available early in pregnancy and should be offered to couples at risk. Mothers carrying a fetus with hydrops fetalis (4α chain deletion) are also at risk for certain maternal complications such as toxemia and postpartum hemorrhage [Wasi et al., 1969].

Thalassemias are more difficult to diagnose or exclude during pregnancy because common nutritional deficiencies alter the MCV. Microcytosis due to iron deficiency is common during pregnancy [Godel et al., 1992; Romslo et al., 1983; Thompson, 1988]. Coexistent folic acid deficiency may increase the MCV. Because exclusion of iron deficiency is important in the evaluation of thalassemia and because iron studies may not reliably exclude iron deficiency during pregnancy, the diagnosis of thalassemia is best made before pregnancy. When a patient with microcytosis presents for genetic counseling during pregnancy or presents for prenatal diagnosis for other reasons and thalassemia has not



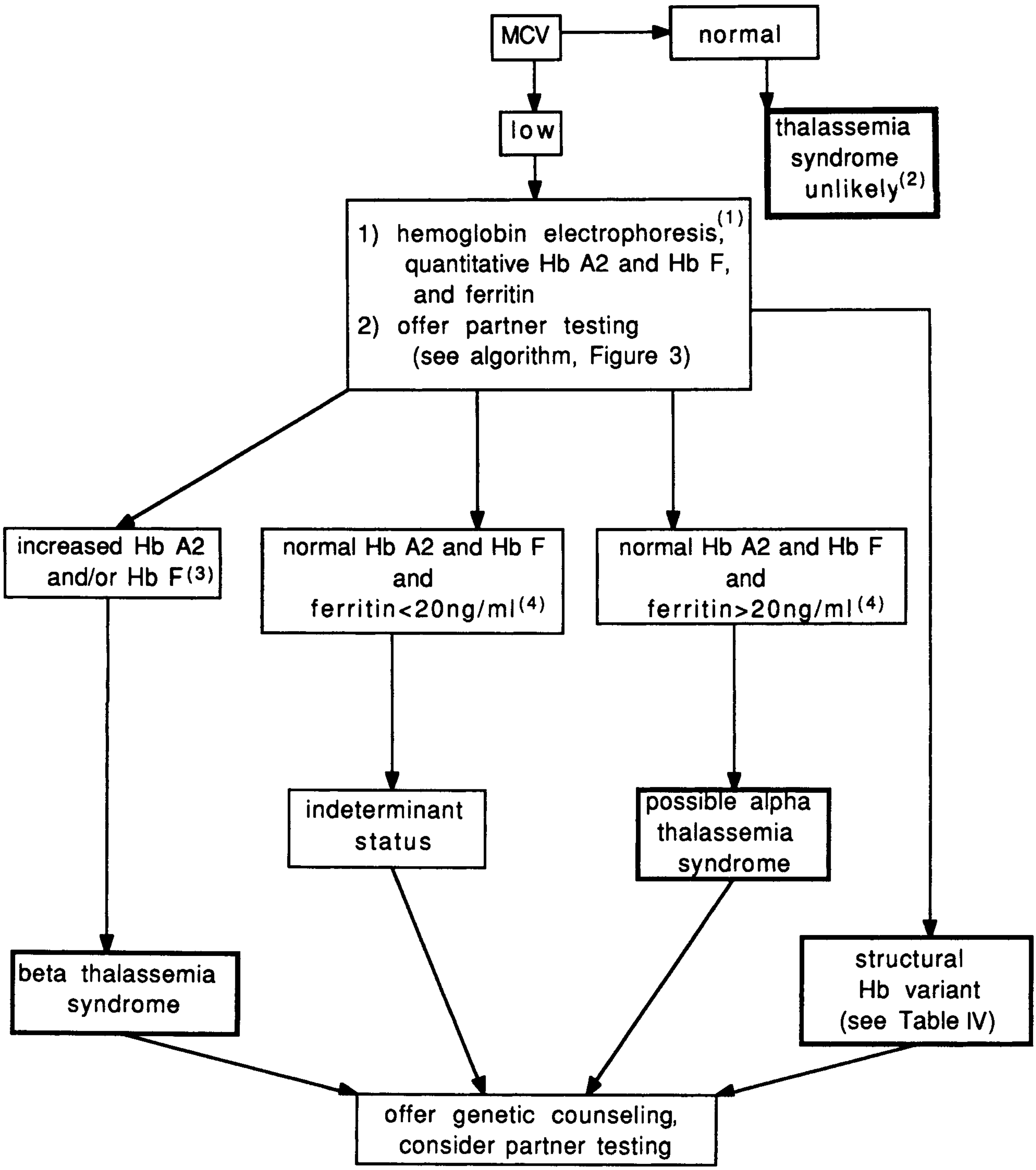
Age-related Normal Values for Hemoglobin, MCV and Ferritin

Age (yr)	Hemoglobin[1] (g/dl) Mean (-2 SD)	MCV[1] (μ^3) Mean (-2 SD)	Ferritin[2] (ng/ml) Reference ranges
1-2	12.0 (10.5)	78 (70)	7-140
2-6	12.5 (11.5)	81 (75)	7-140
6-12	13.5 (11.5)	86 (77)	7-140
12-15			
Female	14.0 (12.0)	90 (78)	7-140
Male	14.5 (13.0)	88 (78)	7-140
15-18			
Female	14.0 (12.0)	90 (78)	12-150
Male	14.5 (13.0)	88 (78)	15-200
Adult			
Female	14.0 (12.0)	90 (80)	12-150
Male	15.5 (13.5)	90 (80)	15-200

[1]From Nathan DG, Oski FA. *Hematology of Infancy and Childhood.* 4th ed. Philadelphia: Saunders, 1993; appendix iii.
[2]From Tietz NW. *Textbook of Clinical Chemistry.* Philadelphia: Saunders; 1986:1824.

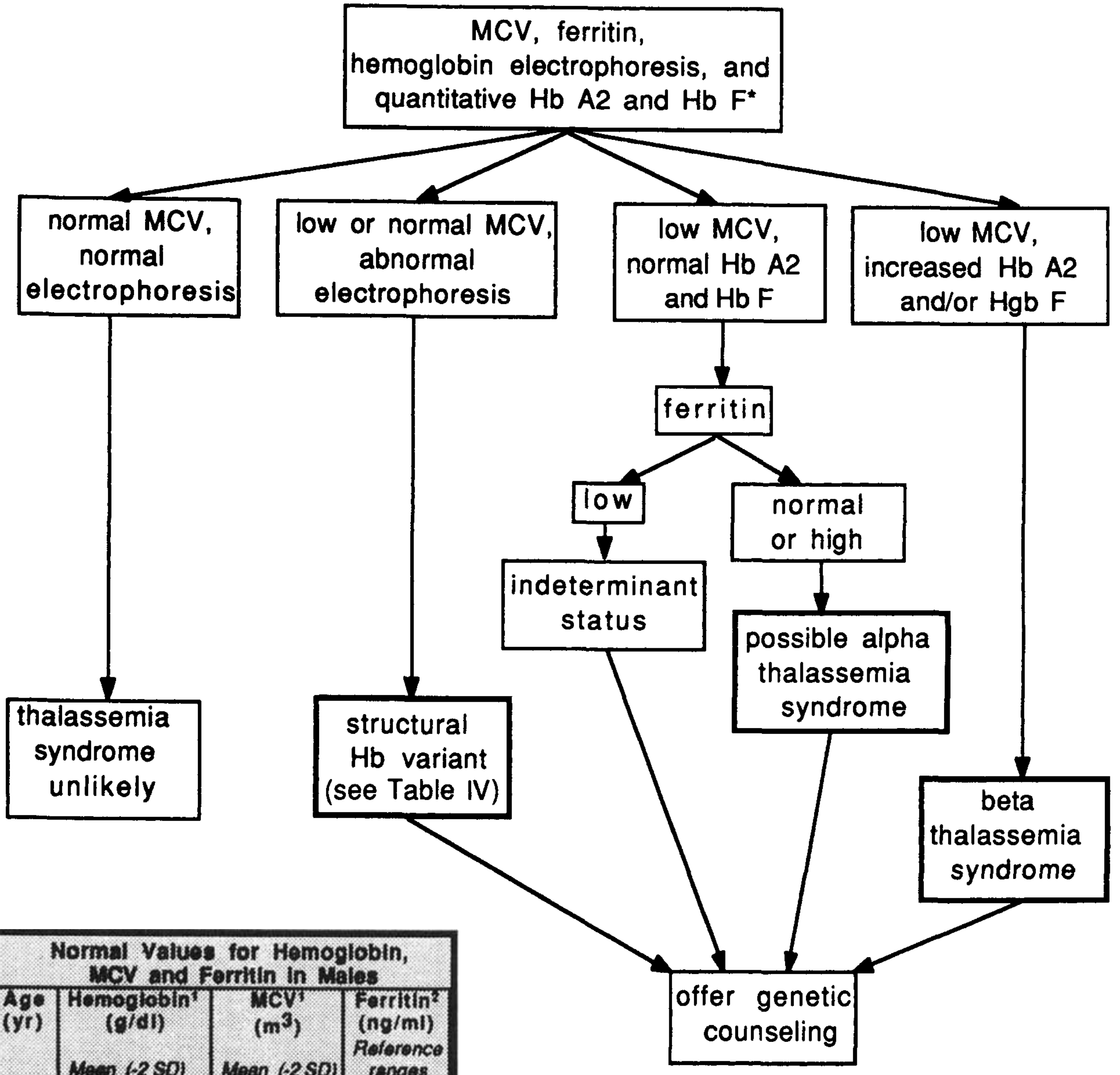
*Iron deficiency may exist with ferritin values up to 100ng/ml. Ferritin is an acute phase reactant and values may be elevated in iron deficiency with coexistent liver disease or inflammation.
**Atypical cases of beta thalassemia and alpha thalassemia silent carrier is not ruled out in this case.

Fig. 1. Algorithm for the detection of thalassemia syndromes using mean corpuscular volume (MCV). (Excluding pregnant women and children less than one year of age.)



(1)Given the need to diagnose expediently during pregnancy, electrophoresis is performed simultaneously with tests to rule out iron deficiency.
(2)Structural hemoglobin variants, e.g., Hb S, E, C, D, or Constant Spring, will often not have a low MCV. In certain ethnic groups, Hb electrophoresis should be considered to detect structural variants.
(3)Hb F may be slightly elevated in normal women during pregnancy.
(4)References- Godel JC, Romslo I, and Thompson WG.

Fig. 2. Algorithm using MCV for the detection of thalassemia syndromes during pregnancy.



Normal Values for Hemoglobin, MCV and Ferritin in Males

Age (yr)	Hemoglobin[1] (g/dl) Mean (-2 SD)	MCV[1] (m^3) Mean (-2 SD)	Ferritin[2] (ng/ml) Reference ranges
15-18	14.5 (13.0)	88 (78)	15-200
Adult	15.5 (13.5)	90 (80)	15-200

[1]From Nathan DG, Oski FA. *Hematology of Infancy and Childhood.* 4th ed. Philadelphia: Saunders, 1993; appendix II.
[2]From Tietz NW. *Textbook of Clinical Chemistry.* Philadelphia: Saunders; 1986:1824.

*Because of the need to detect thalassemia in a timely fashion during pregnancy, it is recommended that all of these screening tests be performed during the initial evaluation.

Fig. 3. Algorithm for the detection of thalassemia syndromes during pregnancy: partner testing.

TABLE II. Age-Related Normal Values for Hemoglobin and MCV*

Age	Hemoglobin (g/dl) Mean (−2 S.D.)	MCV (μ^3) Mean (−2 S.D.)
Birth (cord blood)	16.5 (13.5)	108 (98)
1 to 3 days	18.5 (14.5)	108 (95)
1 week	17.5 (13.5)	107 (88)
2 weeks	16.5 (12.5)	105 (86)
1 month	14.0 (10.0)	104 (85)
2 months	11.5 (9.0)	96 (77)[a]
3 to 6 months	11.5 (9.5)	91 (74)
0.5 to 2 years	12.0 (10.5)	78 (70)
2 to 6 years	12.5 (11.5)	81 (75)
6 to 12 years	13.5 (11.5)	86 (77)
12 to 18 years		
female	14.0 (12.0)	90 (78)
male	14.5 (13.0)	88 (78)
Adult		
female	14.0 (12.0)	90 (80)
male	15.5 (13.5)	90 (80)

*From Nathan and Oski [1993].
[a]In Saarinen and Silmes [1978], the value for MCV −2 S.D. at this age is listed as 84. The other values in this table are more consistent with values reviewed from this and other sources.

been excluded, the algorithms shown in Figures 2 and 3 may be used to identify most couples at risk of having a child affected by thalassemia.

IMPLICATIONS OF DIAGNOSIS

Both α and β thalassemias encompass a broad spectrum of clinical severity. Phenotypic and hematologic *manifestations* of some of the more common syndromes including estimates of clinical severity are presented in Table III. A number of references listed at the end of this paper describe these characteristics in depth. All individuals with thalassemia should be offered health education including family planning, genetics services, and referral to consultants when appropriate for diagnostic evaluation and therapy.

Health Education

Primary care providers or counselors who assume responsibility for education of the individuals with thalassemia should have accurate information about the specific thalassemia syndrome present in the family. Topics to discuss with patients and families should include: (1) adequate information to enable the individual and family to make informed choices about their medical care; (2) discussion of the genetic transmission and spectrum of manifestations of disease in the family, from the benign nature in carriers to potential life-threatening illnesses in homozygotes and some compound heterozygotes; (3) medical management of all forms of thalassemia expected in the individual or family, from no treatment to comprehensive and often intense medical therapy; (4) discussion of the psychological and social implications of the disorder presented in a culturally sensitive manner; (5) reproductive issues, with referral to genetic counseling when appropriate; and (6) information about sources of medical, social, and psychological support.

Genetics Services

Individuals or families with thalassemia should be offered genetic services provided by knowledgeable professionals. Such services include education, genetic counseling, and prenatal diagnosis. Prenatal testing is possible for most couples at risk for having an infant with severe thalassemia. When couples at risk for thalassemia present for prenatal diagnosis, expeditious clinical investigation of mother and partner is necessary to provide timely diagnosis and genetic counseling (Figs. 2, 3). Because of the complicated molecular na-

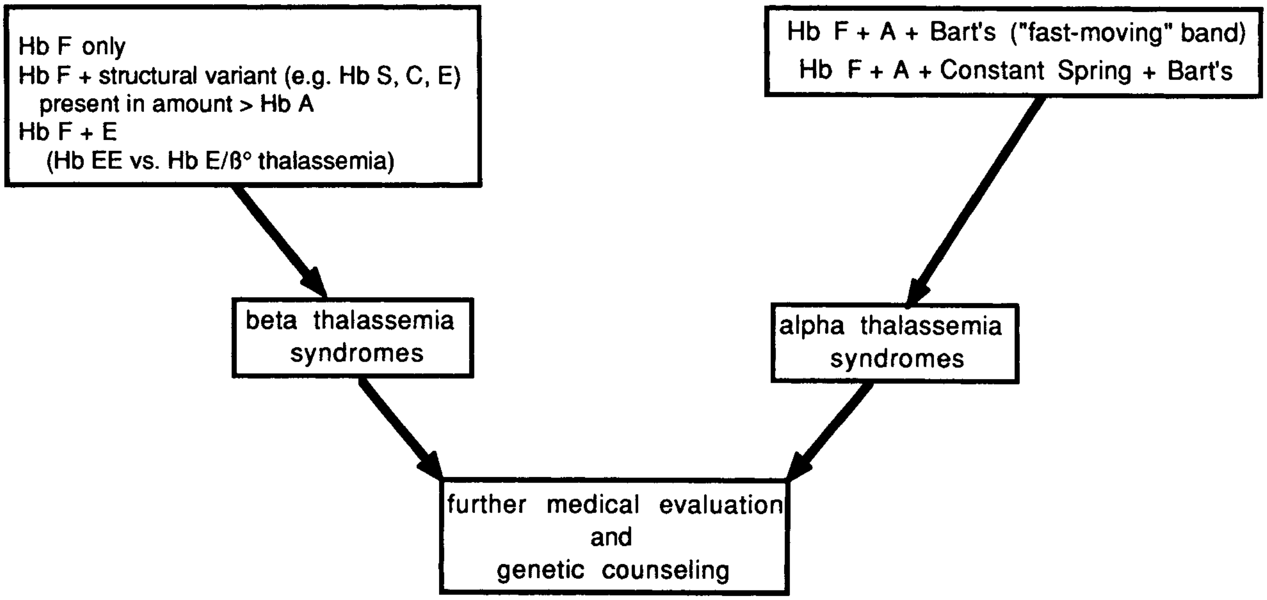


Fig. 4. Newborn screening results requiring follow-up evaluation for thalassemia.

TABLE III. Classification of the Common Thalassemia Syndromes*

		Phenotype		
			Laboratory	
Syndromes	Genotype[a]	Clinical manifestations	RBC indices	Electrophoresis
α thalassemia				
Hydrops fetalis	4 α gene deletions, (–/–)	Usually lethal in utero; implications for maternal health during pregnancy	↓MCV, ↓MCH	No Hb A or F 80–90% Hb Bart's
Hb H disease	3 α gene deletions, (α-/–)	Moderate anemia; usually not transfusion dependent	↓MCV, ↓MCH	Neonate: 10–30% Hb Bart's Adult: 4–20% Hb H
α thalassemia trait (α-thal-1 heterozygote or α-thal-2 homozygote)	2 α gene deletions, ($\alpha\alpha$/–) or (α-/α-)	May have mild anemia	↓MCV, ↓MCH	Neonate: 2–10% Hb Bart's Adult: normal Hb pattern
Silent carrier (α-thal-2 heterozygote)	1 α gene deletion, (α-/$\alpha\alpha$)	None	Normal	Neonate: 0–3% Hb Bart's, Adult: normal Hb pattern
Heterozygous Hb Constant Spring (α-chain termination mutation)	($\alpha\alpha/\alpha^{cs}\alpha$)	May have mild anemia	↓MCV, ↓MCH	~1% Hb Constant Spring
Homozygous Hb Constant Spring	($\alpha^{cs}\alpha/\alpha^{cs}\alpha$)	Mild to moderate anemia; usually not transfusion dependent	↓MCV, ↓MCH	~5% Hb Constant Spring
Hb H with Hb Constant Spring	($\alpha^{cs}\alpha$/–)	Moderate anemia; may be transfusion dependent	↓MCV, ↓MCH	2–3% Hb Constant Spring, 10–15% Hb H
β thalassemia				
β thalassemia major (Cooley's anemia)	(β°/β°), (β°/β^+), or (β^+/β^+)	Severe anemia; usually transfusion dependent	↓MCV, ↓MCH	Absent or very low levels of Hb A present
β thalassemia intermedia	(β°/β°), (β^+/β^+) or, (β°/β^+) This category also includes structural hemoglobin variants co-inherited with thalassemia mutations, e.g., (β^E/β°) and (β^E/β^+)	Moderate to severe anemia; usually not transfusion dependent. (See below for clinical manifestations seen in Hb E/β thalassemia)	↓MCV, ↓MCH	Variable amounts of Hb A present, Hb A_2 and Hb F normal or increased. (See below for patterns seen in Hb E/β thalassemia)
β° or β^+ thalassemia carrier (Heterozygote)	(β/β°) or (β/β^+)	May have mild anemia	↓MCV, ↓MCH	Hb A predominates, increased levels of Hb A_2, normal or increased Hb F
Hb Lepore syndromes (Defective β and δ chain synthesis)	(β^{Lep}/β^{Lep})	Severe anemia; can be transfusion dependent	↓MCV, ↓MCH	Hb F and Lepore
	(β^{Lep}/β)	Mild anemia	↓MCV, ↓MCH	Hb A, A_2, F, and Lepore
Hemoglobin E				
Hb E carrier (Heterozygote)	(β^A/β^E)	None	May have microcytosis	Hb A and E (with amount of Hb A >Hb E)
Hb E syndrome (Homozygote)	(β^E/β^E)	Mild anemia	↓MCV, ↓MCH	Hb E
Hb E/β^+ thalassemia	(β^E/β^+)	Mild to moderate anemia	↓MCV, ↓MCH	HB E and A (with amount of Hb E > Hb A), usually increased Hb F
Hb E/β° thalassemia	(β^E/β°)	Moderate to severe anemia; may be transfusion dependent	↓MCV, ↓MCH	Hb E and increased Hb F

*This table does not address the less common structural globin variants or the unstable thalassemic hemoglobinopathies, both of which can coexist with thalassemia and result in clinical manifestations; nor does it address the multiple molecular point mutations that comprise the β and α thalassemias that can present with variable clinical severity.

[a]β° indicates the absence of β-globin chain production; β^+ indicates severely reduced β-globin chain synthesis; and β or β^A indicates normal production of a structurally normal β-globin chain. β^E indicates the structural globin chain variant of hemoglobin E.

ture and variable clinical expressions of some of the thalassemia syndromes, it is preferable to perform DNA analysis of the parental mutations prior to actual fetal sampling. Diagnostic DNA laboratories with experience in analyzing thalassemia mutations should be consulted before obtaining fetal samples for prenatal diagnosis.

Individuals from ethnic groups at risk for thalassemia are also at risk for structural hemoglobin variants such as Hb S, C, E, D, Lepore, and Constant Spring (Table IV). Compound heterozygotes with a structural variant and thalassemia (for example, Hb S/β thalassemia) may have serious clinical manifestations. Therefore, individuals from ethnic groups at risk for thalassemia should be offered hemoglobin electrophoresis to rule out a structural hemoglobin variant, even if the MCV is normal.

General Therapeutic Considerations

The milder forms of thalassemia usually do not require specialized management. Supplemental iron administration should be avoided unless iron deficiency is proven. In cases with documented iron deficiency, iron therapy should be monitored to avoid excessive treatment. Changes in hemoglobin from the patient's usual baseline level should be evaluated. Falls in the hemoglobin level associated with pregnancy may occasionally precipitate symptomatic anemia.

Moderately severe forms of thalassemia (i.e., Hb H disease, β-thalassemia intermedia, and some Hb E/β thalassemias) require ongoing medical care. Important considerations include administering supplemental folic acid, avoiding oxidant drugs and unnecessary iron, and monitoring for normal growth and development, hypersplenism, and progressive iron overload. Acute exacerbations of anemia require evaluation and support, including transfusions, and may occur with infection, splenic sequestration, aplastic crisis, pregnancy, and folate deficiency. The patient may also develop symptomatic gallstones and/or leg ulcers. Periodic consultation with a hematologist knowledgeable in management of thalassemia is strongly recommended.

TABLE IV. Commonly Encountered Structural Hemoglobin Variants With Implications for the Diagnosis of Thalassemia*

A. Interactions of Structural Variants With β Thalassemia	
Hemoglobin phenotype[a]	Genotype
Hb S	S/S or S/β° thalassemia
Hb SA	S/β^+ thalassemia
Hb C	C/C or C/β° thalassemia
Hb CA	C/β^+ thalassemia
B. Structural Variants Associated With Thalassemia Phenotypes	
Hemoglobin variant	Phenotype
Hb E	β thalassemia
Hb Constant Spring	α thalassemia
Hb Lepore	β thalassemia

*See Table III for a more complete listing of thalassemia syndromes.
[a]Hemoglobins are listed in order of quantitation (e.g., Hb SA indicates that Hb S is present in quantity greater than Hb A).

Therapeutic considerations for severe forms of thalassemia include regular transfusion therapy, iron chelation, and bone marrow transplantation. All patients with these severe forms should be cared for by physicians with expertise in the management of thalassemia and iron overload. Ideally, centers that provide multidisciplinary management for patients with hemoglobin disorders should deliver comprehensive care because proper management dramatically improves longevity and quality of life.

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